In Lori Setton's laboratory at Duke University, researchers hope to combine biological materials with stem cells to regenerate spinal discs.

REGENERATIVE MEDICINE

Rebuilding the backbone

Surgeons can help fix damaged vertebrae, but could an infusion of cells in a bioengineered material grow to replace a damaged spinal column?

BY MIKE MAY

In 2004, 45-year-old Mary, who works in retail in Minnesota, experienced the sudden onset of what she now recalls as “excruciating” neck pain. She endured the pain for seven weeks. When her physician finally ordered a magnetic resonance imaging (MRI) scan, it revealed damage in the middle of her neck. Surgery to open up the spinal canal inside the vertebrae stopped the pain. Then, last year, Mary’s neck started getting stiff, and she began to feel a numbness in her arms. This time, a surgeon fused two of her vertebrae, which has impaired her ability to move her head. “I don’t have the range of motion that I did before the fusion,” she says, “but it’s my new normal.”

Mary is not alone in her spine-related misery. Lower back pain, for example, strikes more than eight out of ten people in their lifetime,

and almost one-quarter of people suffer chronic lower back pain; it becomes a disability for more than 10% of the population¹. Even about 40% of teenagers and children experience lower back pain at some point. Technology under development, however, could repair bad spines without the movement limitations created by bone fusion.

PLACES TO REPAIR

The flexible structure of the spine — there are 24 moving vertebrae with discs between them — provides many points vulnerable to damage. A fall can break your back, but the bones might heal on their own. For example, US goalkeeper Tim Howard broke two bones in his back while playing an English FA Cup football match in 2013, and they were left to heal naturally

because they did not affect the stability of the spinal column.

But if the break puts the mechanical stability of the spine in jeopardy or occurs in a patient at high risk of the bone failing to heal — such as smokers or people with diabetes — then doctors need to act. Iain Kalfas, a spine surgeon at the Cleveland Clinic in Ohio, might apply a bone morphogenetic protein (BMP) to the fracture; the best at accelerating bone growth is BMP-2, which is marketed as Infuse by medical technology company Medtronic, based in Minneapolis, Minnesota.

Growth accelerators cannot always be used, however, because BMP is considered off-limits for some groups, such as cancer patients. “Anything that increases the rate of division of the healing cells might do the same for cancer cells,” explains Michael J. Yaszemski, a spine surgeon at the Mayo Clinic in Rochester, Minnesota. And cost is also an issue. “BMP is

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incredibly expensive right now,” Yaszemski says. A single dose can cost up to US\$6,000, and some treatments require several doses. In addition, BMP is often used in a physician-directed application (‘off-label’ use), which means that health insurance might not reimburse the treatment, leaving the patient to pay the bill. Alternatively, a surgeon can use demineralized bone matrix (DBM). This consists of the organic material in bone, including BMPs and other growth factors. A single dose of DBM costs less than \$800.

The decision to use Infuse or DBM depends on several factors. First, Kalfas says that although DBM increases the potential for bone fusion, Infuse has a better track record, so surgeons prefer Infuse if they suspect that a patient’s bone is less likely to heal. The second issue is cost. If Infuse is not absolutely required, a patient might opt for DBM to save themselves tens of thousands of dollars. Third, most surgeons do not use Infuse in the neck because of the risk of swelling that can inhibit breathing.

Some of the most common back surgeries involve the intervertebral discs. Each disc resembles a tyre with a jelly-like centre known as the nucleus pulposus (see ‘Details in the discs’). The discs serve as shock absorbers between the vertebrae, allowing bending and twisting, and keep the bones separated to allow nerves to run in and out of the spinal canal. When compressed, the nucleus pulposus pushes out and the tyre-like annulus fibrosus prevents a blow out. But the discs can become worn over time or damaged from injury. If they do not keep the vertebrae apart, spinal nerves are compressed, causing the pain that Mary experienced.

Spine surgeries, in order of increasing complexity, range from a discectomy, which removes part of a bulging disc, to a laminectomy — like Mary’s first procedure, which makes room for nerves by removing part of the bone — to a fusion. In a typical fusion, a surgeon inserts two screws in each vertebra to be fused and connects them with titanium rods. “The screws and rods act like a cast,” Kalfas says, “keeping the spine still enough for a bone bridge to form between the vertebrae.” Surgeons have typically triggered the bone growth with a graft from the pelvis, but Kalfas takes a different approach. He starts with a laminectomy, removing bone with a high-speed drill. He collects the pulverized bone material and places it around the vertebrae to be fused, and usually adds DBM to aid bone growth.

FIXING A FLAT

Rather than fusing vertebrae when a disc is damaged, surgeons can replace the disc with an artificial one, but this is much less common. Disc implants are typically made of metal, plastic or a combination of both. But some researchers hope to find a more natural method of repair that does not involve introducing foreign material into the body.

One approach is to inject something into a damaged disc to ‘reinflate’ it. “People are looking for molecules that can induce matrix production — the part that surrounds the cells,” says Zili Wang, a researcher at the Emory Orthopaedics and Spine Center in Atlanta, Georgia. “Traditionally, people have used BMP.” Wang and his colleagues have shown that a molecule called link protein peptide turns on the creation of molecules that make up the matrix in intervertebral discs². Link protein peptide creates the matrix without making as much bone as BMP-2 does, which is beneficial because filling a disc with bone would be like filling a tyre with gravel.

Skin cells could also trigger a fix. “In a mechanically stressed and low-oxygen environment like the spine, dermal fibroblasts turn into cartilage,” says Peter O’Heeron, chief executive of medical technology company SpinalCyte, based in Houston, Texas. Working with Antonios Mikos, a bioengineer at

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Rice University, also in Houston, SpinalCyte has shown that this process works in tissue culture. Studies in rabbits by Howard An, an orthopaedic surgeon at Rush University Medical Center in Chicago, Illinois, showed that injecting these skin cells into the nucleus pulposus created chondrocytes — cartilage cells — in just four weeks. These chondrocytes, like typical cells in the nucleus pulposus, can release matrix materials to “pump up” the disc, O’Heeron says.

But some researchers believe that cells alone are insufficient to induce disc repair. For example, mechanical engineer Lori Setton of Duke University in Durham, North Carolina, points to the work of Daisuke Sakai at Tokai University School of Medicine in Tokyo. The Tokai group implanted mesenchymal stem cells (MSCs) embedded in a collagen gel into degenerated discs in rabbits, and found that the gel enhanced the proliferation and differentiation of the MSCs³.

This work is still in the early stages. “There are lots of injectable biomaterials out there that have gone through clinical trials,” Setton says, “but none that allow cell delivery.” Setton and her colleagues hope to change that, and have tested a modified injectable polyethylene glycol gel to deliver autologous primary disc cells, which come from the patient⁴. The team selected this approach because of a technology called autologous disc chondrocyte transplantation developed by German company Co.don, which showed that autologous chondrocytes can survive and produce matrix when injected into a damaged disc. Setton’s team hopes for even better results by combining the autologous cells with a biological material that provides a place for them to grow.

Some work suggests that allogenic cells, which do not come from the patient, can also be used. For example, Mesoblast, a medical technology company based in Melbourne, Australia, is doing a phase II trial in which it injects damaged discs with allogenic stem cells plus hyaluronic acid, which is essentially a chain of sugar molecules found in many parts of the body, including the skin and joints. Mesoblast reported that interim results from the trial indicated reduced back pain in almost three-quarters of the test patients.

Similarly, TissueGene, a biopharmaceutical company based in Rockville, Maryland, is developing a product called TG-D, which uses genetically engineered allogenic cells to repair discs. TG-D consists of human chondrocytes that produce transforming growth factor β -1, a protein that stimulates the production of more chondrocytes and other cells that add to the disc matrix.

Injecting any kind of cells into a disc causes problems, starting with the needle puncture. “That hole needs to be plugged,” says James Iatridis, a mechanical engineer

DETAILS IN THE DISCS

One of 24 vertebrae designed to give mechanical support to the body and protect the spinal cord.

at Mount Sinai School of Medicine in New York. Iatridis has modified fibrin — a protein involved in blood clotting that is often used as a surgical adhesive or sealant — to make it stiff enough to match the mechanical properties of the annulus fibrosus, and has tested it in tissue culture and animal models as a sealant for needle holes⁵. “This is an injectable sealant that may be very helpful for small defects, and a similar strategy could also work for repairing larger injuries,” he says. “My dream is that a surgeon could use one needle and two syringes to first inject a cellular therapy and then fix the hole to restore the disc to its healthy biomechanical state.”

These treatments might avoid the need for extensive repairs. Younger patients who come in with early signs of disc disease, for example, might benefit from therapies that stop the damage getting any worse and slow disease progression.

CHANGING THE TYRE

Instead of repairing a flattened disc, bioengineers Jason Burdick and Robert Mauck, both of the University of Pennsylvania in Philadelphia, hope to replace it with biological material. “We start with molecules that we know are found in the body,” says Burdick. A good example is hyaluronic acid. With chemical modifications, researchers can turn it into many forms, including a hydrogel — basically a watery gel that is often used in tissue repair.

But designing a new disc requires more than finding the right material. The annulus fibrosus consists of 15–25 concentric rings like those in the trunk of a tree, except these are made of collagen and separated by elastin fibres. The fibres in the layers alternate orientations — those in one ring are angled at about 30 degrees higher than the plane of the disc, those in the next ring are 30 degrees lower than the plane, and so on. This arrangement gives the disc its strength. Mauck, Burdick and their colleague Dawn Elliott of the University of Delaware used electrospinning, which creates fine fibres from a liquid, to make structured scaffolds to grow stem cells in⁶.

The researchers hope to use these techniques to make new discs, which they are now testing in a rat's tail. Mauck sounds a note of caution: “The amount of over-promising that has happened is not good for our field.” So all he says is: “Some aspects are working out well, and other aspects not so well.”

Some of the most advanced disc replacement work comes from Lawrence Bonassar, a biomedical engineer at Cornell University in Ithaca, New York. He and his colleagues have engineered a disc from a ring of annulus fibrosus cells suspended in collagen with a jelly-like centre created from nucleus pulposus cells in alginate, which is another chain of sugars. “We made two tissues and put them together to work in concert,” Bonassar says. His team

A biologically engineered disc (right) has been used to replace a real one (left) in rats, and could eventually be used in humans.

used this structure to replace one disc in a rat's tail, and the tail was fully functional at the end of a six-month study⁷. “We asked if we could replace a healthy disc with ours and still get it to integrate with the surrounding spine and endure the normal loading patterns,” Bonassar explains. The result, he says, was “a resounding thumbs up”.

Bonassar acknowledges, however, that a rat's tail is very different to a human's lower back or neck. The next step is to test the discs in the neck of a dog. This could be used to help dogs, some of which suffer from cervical disc disease, and it may lead to studies in humans. Bonassar is now trying to incorporate cells derived from human bone marrow into his engineered disc as he moves towards human applications.

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FIT FOR PURPOSE

Engineering alone does not create a treatment, however. Once you have developed a biomaterials-based disc, you still need to fit it, so engineers must collaborate with surgeons. “We come up with a crazy idea,” Mauck says, “and the surgeons ask: ‘How would we get that in there without paralysing the person?’” The design needs to meet the mechanical constraints imposed on a disc, as well as the biochemical ones, and must be able to be put in place and kept there.

Keeping a bioengineered disc in place poses a significant challenge. With the rat's tail, Bonassar's team made the disc about twice as thick as the opening, spread the vertebrae, inserted the disc and released the vertebrae, which compressed the disc to hold it in place. In larger animals, though, stabilization might require glue or sutures. Research and testing

will be needed to determine the best way to stabilize a bioengineered disc.

A huge amount of research is underway to develop replacement discs made from biological materials, and much more needs to be done before they can even be tested in humans. Is it worth the effort when metal or plastic discs already exist? There are several reasons why it is. “An artificial disc is on borrowed time as soon as you put it in,” says Bonassar. His bioengineered discs, by contrast, get stronger and stiffer over time because of the growth and proliferation of the cells. Also, if one of his discs is damaged, the body will absorb any pieces because they are made of natural materials.

In addition, bioengineered parts could provide more realistic replacements that perform better than metal ones, as they can be made from materials that closely resemble the natural structure. Furthermore, bioengineered parts might interact better with the surrounding tissues, given their similar constituents.

Even now, some of the most advanced treatments for spinal column injuries use natural materials. In the future, even more biology-based materials will be used, allowing part of the backbone to be rebuilt from scratch. The rejuvenating abilities of biology should never be ignored. ■

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